Vol. 123, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1984 Pages 312-316

DOPAMINE RECEPTORS IN RAT PITUITARY AND ESTRADIOL-INDUCED PITUITARY TUMOR: EFFECT OF CHRONIC TREATMENT WITH BROMOCRIPTINE

Thérèse Di Paolo and Pierre Falardeau

Department of Molecular Endocrinology, Le Centre Hospitalier de l'Université Laval, Québec, G1V 4G2, Canada

Received July 6, 1984

SUMMARY: We have investigated dopamine (DA) receptors in estradiol-induced PRL-secreting pituitary tumors and intact pituitary tissue. Female rats were injected at 3-week intervals with 2 mg estradiol valerate (EV) or with diluent. After 21 weeks, adenomatous changes in the pituitary gland of EV-treated rats were seen and plasma PRL concentrations reached 2 µg/ml. Bromocriptine (2.5 mg/kg) was then administered for 1 month to half of the control rats and half of the rats bearing tumors. Anterior pituitary weight was increased in EV-treated rats compared to controls while the affinity and the density of DA receptors as assessed by [^{3}H]spiperone binding remained unchanged. Bromocriptine (CB-154) induced a 70% decrease in the density of DA receptors without any change in affinity both in normal pituitaries and in tumors. Concurrently, the elevated plasma concentrations of PRL in the tumor bearing rats were decreased to control values following the CB-154 treatment. Our data suggest that rats with primary estrogen-induced PRL secreting tumors have normal pituitary DA receptors.

In man, prolactin-secreting tumors represent the majority of hormone-secreting pituitary adenomas and prolactin (PRL) hypersecretion is present in 60-70% of patients bearing pituitary tumors previously classified as functionless adenomas (1,2). Bromocriptine (CB-154), a dopamine (DA) agonist, is commonly and succesfully used for long-term treatment of human prolactinomas (3, 4). However, little data is yet available on the effects of long-term CB-154 administration on DA receptors in pituitary tumors. We have thus studied DA receptors in rats bearing estradiol-induced PRL-secreting pituitary tumors which have recently been characterized as a model for human prolactinoma (5).

MATERIALS AND METHODS

Adult female Sprague-Dawley rats (Charles River CD strain) weighing 200-250 g were housed two per cage and maintained at 22-23°C on a 14:10 hours light dark cycle (lights on from 0.500 to

0006-291X/84 $1.50

19.00 hours) and received rat chow and water ad libitum. Rats were given 2 mg 17β-estradiol valerate (EV), s.c. at 3-week intervals while control rats received the vehicle, 0.2 ml sesame oil. After 21 weeks, half of the animals were administered bromocriptine (2.5 mg/kg, s.c. b.i.d.) or the vehicle (1% gelatine in 0.9% saline solution) for one month. Animals were sacrificed by decapitation on the third morning after their last bromocriptine injection. The anterior, intermediate and posterior lobes of the pituitary gland were dissected before being frozen in dry ice. Tissues were kept at -90°C until assayed.

Trunk blood was collected into heparinized tubes and plasma was separated by centrifugation at 4000 g for 10 min and kept at -20°C until assayed for prolactin. Blood samples were also collected from the tail veins during induction of the tumors. Prolactin was measured in duplicate by double-antibody radioimmunoassay (6) using rat prolactin I-5 and rabbit antisera (anti-prolactin-S-8) kindly supplied by Dr. S. Raiti of the National Hormone and Pituitary Program, USA.

Intact and adenomatous anterior pituitary tissue preparation for dopamine receptor assay was as follows. Tissue was thawed, weighed and homogenized, using a glass-teflon homogenizer in 100 vol/wet weight of tissue of 0.25M sucrose, 25 mM Tris-HCl, 2 mM $MgCl_2$, pH 7.4 at 0-4°C. This homogenate was centrifuged at 100 x g for 10 minutes in conical tubes, the pellet of this centrifugation is discarded, and the supernatant centrifuged at 30,000 x g for 20 min. The pellet was resuspended in the same buffer without sucrose and centrifuged 15 min at 30,000 x g. The final pellet was resuspended in 25 mM Tris-HCl, 2 mM $MgCl_2$, 10 µM pargyline, 0.1% ascorbic acid, pH 7.4 and preincubated 10 min at 25°C before binding assay.

Dopamine receptors were assayed using 10 concentrations of [^{3}H]spiperone and 1 µM (+)butaclamol was used to estimate non-specific binding. [^{3}H]spiperone (20-25 Ci/mmole) was incubated in duplicate for 60 min at 25°C in a total volume of 0.5 ml: 200 µl of tissue suspension (dilution 50 vol/wet weight of tissue corresponding to approximately 1 mg of protein/ml), 50 µl of [^{3}H]ligand (0.05-1.0 nM), 50 µl of (+)butaclamol or ascorbic acid 0.01% and 200 µl of buffer. At the end of incubation, samples were diluted with 3 ml of cold washing buffer and rapidly filtered through Whatman (GF/C) glass fiber filters under reduced pressure. Filters were quickly washed with aliquots (3 x 5 ml) of washing buffer. The radioactivity trapped on the filters was measured by liquid scintillation spectroscopy at an efficiency of 30%. Protein concentration was determined by the method of Lowry (7). Dopamine receptors in the anterior pituitary of EV-treated rats were assayed individually while pooled tissue of 2-5 animals were used in the other groups of animals. Anterior pituitary weight and plasma prolactin concentrations were measured in individual animals. Each group contained 20 rats and results shown are the mean ± SEM. The experiment was repeated twice. Statistical significance was measured according to the multiple-range test of Duncan-Kramer (8). [^{3}H]spiperone was from New England Nuclear Corp. while (+)butaclamol (Ayerst) and bromocriptine (Sandoz) were kindly supplied by these companies.

RESULTS

After 21 weeks of EV treatment, plasma PRL concentrations measured in blood collected from the tail vein of treated animals reached concentrations as high as 1800 µg/ml, as shown in Table 1.

Table 1. Effect of chronic estradiol valerate (EV) and bromocriptine (CB-154) treatments on plasma prolactin levels, intact pituitary and tumor weights and dopamine receptors

Group	PRL	Anterior pituitary weight	Anterior pituitary [^{3}H]spiperone binding	
	(ng/ml)	(mg)	K_D (nM)	B_{max} (fmole/mg of protein)
C	60 ±22	21.74 ± 1.57	0.198 ±0.081	108.8 ±2.3
CB-154	28 ±16	17.68 ± 1.39	0.220 ±0.042	29.5 ±2.5b
EV	1846 [1]±288; 3569 [2]±615b,e	186.02 ±26.91b	0.204 ±0.047	93.6 ±8.8
EV + CB-154	1458 [1]±221; 167 [2]± 24c,e	61.43 ± 7.46c,a,d	0.350 ±0.049	31.4 ±3.9b,c

[1]measured in blood collected from the tain vein after 21 weeks of EV treatment.
[2]measured in the trunk blood at sacrifice of the animals after 25 weeks of EV treatment.

a, $p < 0.05$ and b, $p < 0.01$ vs C
c, $p < 0.01$ vs EV
d, $p < 0.01$ vs CB-154
e, $p < 0.01$ vs PRL measured after 21 weeks of EV

These animals were then divided in two troups one vehicle-treated and the other treated for one month with bromocriptine. In rats receiving the EV treatment without bromocriptine plasma prolactin concentrations continued to increase while in those receiving bromocriptine a sharp decrease in prolactin concentrations was observed. Rats not receiving the EV valerate treatment were included for comparison. From Table 1, we observe that the EV treatment induced large increases of PRL concentrations and that bromocriptine was able to reverse this effect and to decrease the plasma PRL concentration back to control values. Rats receiving only the CB-154 treatment show a non-significant decrease in plasma PRL concentrations. The CB-154 treatment had no effect on intact pituitary weight while it significantly reduced the weight of estradiol-induced pituitary tumors. Dopamine receptors were assessed with [^{3}H]spiperone binding. The density (Bmax) and affinity (K_D) for [^{3}H]spiperone binding remained unchanged in EV-treated rats compared to controls. Bromocriptine treatment induced a similar decrease of the density of dopamine receptors in the intact and adenomatous pituita-

ry while it did not influence the affinity of DA receptor for the DA radioligand. Similar results are obtained when the density of DA binding sites is expressed per mg of tissue (data not shown).

DISCUSSION

The animal model of pituitary tumor used in this study has been recently investigated (5). As assessed by plasma prolactin concentrations, Casanueva et al. (5) have shown a defective central nervous system dopaminergic function in rats with estrogen-induced pituitary tumors and observed a decrease in the dopamine concentration of the median eminence. We observe similar results and, in addition, we have shown a decrease in the dopamine concentration in the anterior pituitary of EV-treated rats (9). No data was available on the DA receptors in these tumors. We observe that the density and the affinity of [^{3}H]spiperone for the DA receptors in the pituitary tumors is unchanged compared to intact pituitary tissue while the weight of the anterior pituitary is increased about ten-fold.

In agreement with Casanueva et al. (5), we observed that PRL concentrations, elevated in the tumor bearing rats, were decreased to control values following the bromocriptine treatment. Furthermore, we also noted that the ergot treatment decreased the anterior pituitary tumor weight. Similar results have been observed in humans where treatment with bromocriptine normalizes plasma PRL levels and has also been shown to lead to tumor regression (3, 4, 10). Chronic bromocriptine treatment induced a significant decrease in the number of dopamine binding sites without any change in their affinity both in intact pituitaries and in the tumors. Our data suggest that rats with primary estrogen-induced PRL-secreting tumors have normal pituitary DA receptors. In addition, our results indicate that chronic CB-154 treatment of prolactinomas may modify the number of DA receptors without affecting the responsiveness of tumoral mammotrophs to dopaminergic agents.

ACKNOWLEDGEMENTS

We thank Michel Daigle for his excellent technical work. This research was supported by the National Cancer Institute of Canada.

REFERENCES

1. Franks, S., Nabarro, J.D.M., and Jacobs, H.S. (1977) Lancet 1, 8015, 778-780.
2. Frantz, A.G., Kleinberg, D.L., and Noel, G.L. (1972) Rec. Progr. Horm. Res. 28, 527-590.
3. Lancranjan, I. (1981) J. Neural Transm. 51, 61-82.
4. Thorner, M.O., Flückiger, E., and Calne, D.B. (1980) Bromocriptine: a clinical and pharmacological review. New York: Raven Press.
5. Casanueva, F., Cocchi, D., Locatelli, V., Flauto, C., Zambotti, F., Besetti, G., Rossi, G.L. and Müller, E. (1982) Endocrinology 110, 590-599.
6. Odell, N.D., Rayford, P. and Ross, G.T. (1967) J. Lab. Clin. Med. 70, 973-979.
7. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
8. Kramer, C.Y. (1956) Biometrics 12, 307-310.
9. Di Paolo, T., Falardeau, P., Pelletier, G. and Barden, N. 7th Int. congress of Endocrinology, Abst #484, Quebec, Canada. 1984.
10. Thorner, M.O., Martin, W.H., Rogol, A.D., Morris, J.L., Perryman, R.L., Conway, B.P., Howards, S.S., Wolfman, M.G. and MacLeod, R.M. (1980) J. Clin. Endocrinol. & Metab. 51, 438-445.